REMARKS/ARGUMENTS

Amendments to the Specification

The Examiner pointed out the omission of a Sequence ID NO. on page 59 and pointed out the misuse of several trademarks. In response, Applicants have hereby amended the specification to correct these errors. The sequence ID No. has been inserted, and several trademark indications and generic names have been inserted.

The Examiner objects to the conclusions section and suggests that deleting the conclusions section would overcome this objection. Applicants choose not to delete the conclusions section.

The Examiner pointed out that some of the ATCC deposit numbers were missing. They have now been included in the Specification.

Amendments to the Sequence Listing

The Examiner pointed out that the amino acid sequence EQKLISEEDL on page 59 requires a sequence listing. The Sequence Listing has been amended as requested. Applicant requests that the substitute Sequence Listing be entered into the application. The undersigned states that no new matter is included in this Sequence Listing or in the amendments to the specification above.

Amendments to the Claims

In addition, Applicants have amended the claims. Applicants believe that the previous claim set was fully supported by the specification of the application as filed. However, to hasten an agreement with the Examiner, and to reduce the number issues before the Examiner, Applicants have inserted language to state where maturation of the protein is accomplished, and have included the language of dependent claim 16 as a final step in claim 15.

Rejection of Claims 15-17 under 35 U.S.C. 112, second paragraph

Claims 15-17 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

a) The Examiner states that it is unclear in (d)(iii) where and under what conditions the preproprotein can be processed into the mature polypeptide.

Claim15 has been amended to recite "in the endoplasmic reticulum and/or golgi body of the host cell". The use of a signal peptide together with the language of the claim "allowing the preproprotein to be processed …" can only be interpreted to mean that the maturation is an *in vivo* process in the host cell, and not done by one skilled in the art. Therefore, this inclusion is not considered to be a limiting amendment. Accordingly, applicants respectfully request reconsideration and withdrawal of this ground of rejection.

Applicants have further amended claim 15 to include the limitation(s) of claim 16. This amendment is made only to reduce the number of possible issues before the Examiner. This amendment is not made in response to any objection or rejection raised by the Examiner.

The language of claim 16 inserted into claim 15 is amended to state that the two copies of the mature protein bond to each other.

Claim 17 is objected to for recitation of "a derivative of either the antibody or the Fab fragment. Applicants respectfully traverse this rejection.

The term "derivative of" is used in the same sense as one skilled in the art typically uses it. In the context of this method claim, the derivative must be designed in advance, because the process ends with formation of a multimeric protein. Any derivative can be made, if it is encoded by the nucleotide sequence that encodes one or both of the two peptides of interest. The derivation can be due to a deletion, substitution, or addition to any part of the molecule that a person skilled in the art wants to change. The invention of claim 17 is not concerned with particular derivatives. It is concerned with a novel process by which multimeric proteins can be made. Any antibody or Fab fragment or derivative that is made by the process of claim 17 would be within the scope of this claim. Accordingly, applicants request reconsideration and withdrawal of this rejection.

Characterization of the Invention

The present invention was made specifically to get around the work of Hiatt et al. at Scripps Institute, which enables one to make antibodies in plants.

Hein, M. B., Hiatt, A., **US 6,417,429**, July 9, 2002, Transgenic plants expressing assembled secretory antibodies, Scripps Research Inst. (Antibodies).
Hein M. B., Hiatt, A. C., Fitchen, J. H., **US 6,440,419**, Aug 27, 2002, Novel epithelial tissue targeting agent, Epicyte Pharmaceutical Inc.

Hein, M. B., Hiatt, A., Ma, J. K-C, **US 5,959,177**, Sept 28, 1999, Transgenic plants expressing assembled secretory antibodies, Scripts Research Institute.

Hein, M. B., Hiatt, A. C., Fitchen, J. H., **US 6,251,392**, June 26, 2001, Epithelial cell targeting agents, Epicyte Pharmaceuticals Inc., (targeting for anti-cancer-antibody).

Hein, M. B., Hiatt, A., Ma, J. K-C., **US 5,959,177**, Sept 28, 1999, Transgenic plants expressing assembled secretory antibodies, Scripps Research Inst. (Antibodies).

Hiatt, A. C., Hein, M. B., **US 5,202,422**, April 13, 1993, Compositions containing plant-produced glycopolypeptide multimers, multimeric proteins and method of their use. Scripps Research Inst. (Antibodies).

Hiatt, A. C., Hein, M. B., **US 5,639,947**, June 17, 1997, Compositions containing glycopolypeptide multimers and methods of making same in plants. Scripps Research Inst.(Antibodies).

Hiatt, A. C., Hein, M. B., Fitchen, J. H., **US 6,045,774**, April 4, 2000, J chain polypeptide targeting molecule linked to an imaging agent, Epicyte Pharmaceuticals, (anti-cancer antibodies).

Hiatt, A. C., Hein, M. B., Fitchen, J. H., **US 6,391,280**, May 21, 2002, J chain polypeptide targeting molecule linked to an imaging agent, Epicyte Pharmaceuticals, (anti-cancer antibodies)

Hiatt, A. C., Ma, J. K-C., Lehner, T., Mostov, K. E., **US Patent 6,046,037**, April 4, 2000, Method for producing immunoglobulins containing protection proteins in plants and their use, Planet Biotechnology, (antibodies).

However, the invention is applicable as well for use in animal cells. Both Hiatt and Cabilly use a process of transforming said single host cell with a first DNA sequence encoding at least the variable domain of the immunoglobulin heavy chain and a second DNA sequence encoding at least the variable domain of the immunoglobulin light chain, and independently expressing the first DNA sequence and the second DNA sequence so that the immunoglobulin heavy and light chains are produced as separate molecules in the transformed single host cell.

The present invention enables one to make antibodies and Fab fragments, but includes a wider universe of possibilities. The process of the present invention transfects or transforms a single host cell with only a single gene that encodes both heavy chain and light chain of the antibody and expresses them under the same promoter. Note the differences. This is made possible by use of a propeptide which allows and facilitates proper folding of the preproprotein so that the heavy and light chain are joined in the proper conformation, not as separate molecules, but as one molecule. The propeptide is then removed by enzymes in the ER, leaving a mature protein, which consists of a heavy and a light chain stabilized by a disulfide bond. Two identical mature proteins in contact with each other form a basic immunoglobulin unit having two identical heavy chains and two identical light chains.

Applicants consider the closest prior art to be the method used by Cabilly for animal cells, or Hiatt for plants. In either of these methods the heavy and light chains are encoded on different genes, expressed in a dually transgenic host cell. In the case of Hiatt et al, two transgenic plants must then be crossbred to make cells that express both genes.

Rejection of Claims under 35 U.S.C. 102

Claim 15 is rejected under 35 U.S.C. 102(a) as being anticipated by Francois et al. Francois is said to make six copies of a defensin protein as a single cleavable protein intermediate. The Examiner reminds applicants that because the claim recites "comprising" language, the artificial polynucleotide encoding any "peptide of interest" is encompassed within the scope, and is thus anticipated by Francois. Applicants respectfully traverse this rejection.

It is necessary to look at the function of the propeptide in the process steps. The propeptide made in claim 15 is not merely a cleavage site. A propeptide assists in conformational and proximal positioning of the two peptides of interest and is then removed. The cleavage site of Francois et al. does not have an Assistive function and is not removed, only cleaved. A cleavage site is just a position recognized by a cleavage enzyme. No amino acids are removed during further processing. Not all "peptides of interest" in the universe are within the scope of claim 15. Only those that form a stable intermediate with the help of a propeptide are within the scope of claim 15. For example, the heavy chain and light chain of any antibody from a single fusion gene will form a stable intermediate with the help of a propeptide that is positioned between them although nature designed the heavy and light chains to be encoded on different genes. Accordingly, applicants respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Claims 15-17 are rejected under 35 U.S.C. 102(a) as being anticipated by Wu et al. Applicants respectfully traverse this rejection.

Wu makes a "single-chain antibody" which can cross pair to form a diabody. The method of Wu et al. is to make single-chain antibodies with linkers of 18 or 8 amino acids. The single-chain antibodies of Wu et al. tended to form multimers, probably by "misassembly" wherein a variable region of one single-chain interacts with a variable region of another single chain. Clearly multimerization was not considered to be a desirable characteristic.

The method of claim 15 would not produce misassembly or cross-assembly of variable regions. Therefore, it is not anticipated by Wu et al. In Claim 15, 16 or 17 the preproprotein is assembled into an intermediate with disulfide linkage between the heavy and light chain of the same fusion product. After maturation in which the propeptide and signal peptide are removed, this heavy chain/light chain subunit will come together with another heavy chain/light chain subunit to form a multimer. Because the propeptide guides the assembly of a heavy chain/light chain subunit, no cross-assembly or misassembly can occur. Since no cross-assembly can occur in the method of the present invention, Wu et al. is not an anticipation of the present method, and the resulting multimers are not the same as those taught by Wu et al.

Claim 15 is rejected under 35 U.S.C. 102(b) as being anticipated by Jostick. Applicants respectfully traverse this rejection.

Jostick teaches binding an IL-6 to the Fc region of an IgG molecule, apparently for the purpose of being able to isolate pure IL-6 by cleavage of the thrombin cleavage site. Thrombin recognizes the consensus sequence Leu-Val-Pro-Arg-Gly-Ser, cleaving the peptide bond between Arg and Gly. Jostick is not concerned with stable conformational pairing of two peptides of interest to allow formation of multimers.

The invention of claim 15 makes use of a nucleotide sequence that encodes a propeptide. The nucleotide sequence that encodes the propeptide is part of a single open reading frame that contains both heavy and light chain coding sequences. The propeptide assists in forming a conformational and proximal association between the two peptides of interest, which results in a stable intermediate. The propeptide is then removed, not merely cleaved. Because folding, maturation and multimerization take place in the endoplasmic reticulum, no *in vitro* processing is required. The end result is a fully mature multimeric protein. The multimeric protein made by the process of claim 15 is defined by the folding of the two peptides of interest into a stable conformational intermediate with the assistance of the propeptide (see page 32 of the specification). For this reason, not just any two peptides of interest fit the scope of claim 15. Accordingly, applicants request reconsideration and withdrawal of this ground of rejection.

Rejection of Claims under 35 U.S.C. 103

Claims 15 – 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jostick in view of Cabilly and Sawai. This rejection is respectfully traversed.

The invention of claim 15 makes use of a nucleotide sequence that encodes a propeptide. The nucleotide sequence that encodes the propeptide is part of a single open reading frame that contains both heavy and light chain coding sequences. The propeptide assists in forming a conformational and proximal association between the two peptides of interest, which results in a stable intermediate. The propeptide is then removed, not merely cleaved. Because folding, maturation and multimerization take place in the endoplasmic reticulum, no *in vitro* processing is required. The end result is a fully mature multimeric protein. The multimeric protein made by the process of claim 15 is defined by the folding of the two peptides of interest into a stable conformational intermediate with the assistance of the propeptide (see page 32 of the specification). For this reason, not just any two peptides of interest fit the scope of claim 15.

Jostick teaches binding an IL-6 to the Fc region of an IgG molecule, apparently for the purpose of being able to isolate pure IL-6 by cleavage of the thrombin cleavage site. Thrombin recognizes the consensus sequence Leu-Val-Pro-Arg-Gly-Ser, cleaving the peptide bond between Arg and Gly.

The addition of a thrombin cleavage site cannot substitute for a preproprotein. The preproprotein by definition is an arrangement of peptides having a signal peptide that precedes a proprotein in the arrangement. A proprotein is defined as containing a propeptide in the arrangement. There is no signal peptide in the Jostick arrangement. The thrombin cleavage site cannot substitute for and does not have the properties of a propeptide. The propeptide would allow the two peptides of interest to form a conformational and proximal association between them. Jostick was not interested in such an arrangement. Jostick was only interested in a method of making IL-6 that would allow for ease of isolation. Jostick does not teach the use of a propeptide to obtain a conformational intermediate. Further, the thrombin cleavage site is merely cleaved, leaving extra amino acids on both peptides. The propeptide is removed by enzymes in the endoplasmic reticulum.

Cabilly discloses a method for independently expressing a first DNA sequence and a second DNA sequence so that the resulting immunoglobulin heavy and light chains are produced as separate molecules in a transformed single host cell. This method would not combine with Jostick, which used a single-gene arrangement.

Sawai also produces a heavy chain and light chain independently of each other. This method would not combine with Jostick, which used a single-gene arrangement. The Cabilly and Sawai references teach essentially the same two-gene technology.

There is no statement in any of the references, nor does the Examiner state that that a reference suggests a way to combine and divert the method of either Cabilly or Sawai with Jostick to obtain a one-gene technology for making the multimers of the present invention or even of Jostick. The method of Jostick does not include the making of a stable conformational intermediate between two peptides as a step subsequent to translation. It is not necessary. Accordingly, applicants respectfully request reconsideration and withdrawal of this ground of rejection.

The present invention was made specifically to get around the work of Hiatt, but includes a wider universe of possibilities. The process of the present invention can make the same antibodies but requires only a single gene that encodes both heavy chain and light chain of the antibody and expresses them under the same promoter. This is possible by use of a propeptide which allows and facilitates proper folding of the preproprotein so that the heavy and light chain are joined in the proper conformation. The propeptide is then removed by enzymes in the ER, leaving a mature protein. Two identical mature proteins in contact with each other form a basic immunoglobulin unit having two identical heavy chains and two identical light chains.

Claims 15-17 are rejected under 35 U.S.C. 103(a) over Cabilly, Jostick and Sawai and further in view of Inoue.

Inoue teaches using a cDNA encoding the L chain and Fd' fragment of a mouse/human anti-u-Pa antibody in a *Bacillus brevis* expression vector. Each of the two cDNAs is directly fused with a signal-peptide-encoding sequence of the *mqp* gene, and placed tandemly downstream from the *cwp* promoter. pNU-Fab', pNU-scFab'1 and pNU-scFab'2 contain the two cDNAs connected by a linker sequence encoding a peptide linker of 17 and 24 amino acids respectively. Further, the linker plays no part in conformational folding of the VL and VH peptides.

Clearly, Inuoe does not teach a method for making a multimer. While the disulfide bond between the L and Fd' chains was formed efficiently, as determined by reducing and non-reducing SDS-PAGE, the Fab' produced by *B. brevis* did not form F(ab')$_2$ in the culture medium.

For the reasons given above for Jostick, Cabilly and Sawai and because the method of Inuoe contributes nothing more, and because Inuoe further does not teach a process capable of making a multimer, applicants respectfully request reconsideration and withdrawal of this ground of rejection.

Jostick uses a linker arrangement to make peptides that are similar to that of Inuoe. Cabilly and Sawai both use a two-gene method with each gene being independently expressed. It is impossible to combine Jostick and Inuoe with Cabilly and Sawai. They are clearly different methods that can lend nothing to the other, and the Examiner has not stated any teaching within any of these references that would suggest a combination. For this reason also, applicants respectfully request reconsideration and withdrawal of this ground of rejection, or in the alternative, a statement of the relevance of one reference to the other.

In light of the above amendments and remarks, Applicants believe that the application is in condition for allowance. Any fees required in addition to those mentioned specifically above may be charged to deposit account 500933.

Respectfully submitted,

Dated: ___November 7, 2006______ By: ___/Thomas Gallegos/_______
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